Plasma folate concentrations are associated with depressive symptoms in elderly Latina women despite folic acid fortification[1–3]

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ABSTRACT

Background: A relation between low folate status and depression has been recognized since the 1960s. Since 1998, flour in the United States has been fortified with folic acid, and the prevalence of folate deficiency has decreased dramatically.

Objective: We investigated whether, in this era of folic acid fortification, low folate status is a determinant of depressive symptoms in a cohort of elderly Latinos (aged ≥60 y) participating in the Sacramento Area Latino Study on Aging (SALSA).

Design: In a cross-sectional logistic regression analysis of data from SALSA ($n = 627$ M, 883 F), odds ratios (ORs) were ascertained for elevated depressive symptoms [Center for Epidemiologic Studies Depression Scale (CES-D) score ≥16] among tertiles of plasma folate. Depressive symptoms were assessed by using the CES-D. Plasma folate concentrations were determined by radioassay.

Results: The prevalence of folate deficiency (plasma folate ≤ 6.8 nmol/L) in the SALSA population was <1%. For men, no significant association between folate tertile and high CES-D score was observed. The adjusted OR for high CES-D score in women in the lowest tertile of folate was 2.04 (95% CI: 1.38, 3.02), which was significantly different from that in women in the highest tertile of folate ($P < 0.001$).

Conclusion: These data indicate that, despite folic acid fortification, low folate status is associated with depressive symptoms in elderly Latina women (but not elderly Latino men). *Am J Clin Nutr* 2004;80:1024–28.

INTRODUCTION

It has long been recognized that folate deficiency can contribute to the symptoms of clinical depression (1–4). Moreover, the efficacy of antidepressant medications is influenced by folate status and may be enhanced by folic acid supplementation (1–4). Folate is required for the de novo synthesis of methionine and the subsequent synthesis of S-adenosylmethionine (SAM). SAM serves as the universal methyl donor in a variety of methylation reactions, including those involving DNA, RNA, membrane phospholipids, and neurotransmitters. SAM also was shown to have antidepressant properties (5). Accordingly, it was suggested that the association between folate deficiency and depression is mediated by low cellular SAM concentrations and the consequent inhibition of SAM-dependent methylation reactions in the central nervous system (6).

As of 1 January 1998, the US Government mandated the fortification of the food supply with folic acid to lower the incidence of neural tube birth defects (eg, spina bifida and anencephaly). Reports have indicated that the folic acid fortification program has been successful in reducing both the prevalence of folic acid deficiency in the general population (7, 8) and the incidence of neural tube birth defects (9). Because of the success of the folic acid fortification program, we were interested in investigating whether folate status is associated with depressive symptoms. We addressed this question in a cross-sectional analysis of data from the Sacramento Area Latino Study on Aging (SALSA), a community-based study of physical and cognitive functioning in a cohort of elderly (aged ≥60 y) Latinos (10).

SUBJECTS AND METHODS

Subjects

Subject recruitment and study procedures were approved by the University of California, Davis, Human Subjects Review Committee, and written informed consent was obtained from all study participants. A representative sample of community-dwelling elderly (aged ≥60 y) Latinos residing in Sacramento, CA, and the surrounding Northern California communities was recruited over a period of 1.5 y beginning in February 1998 (after folic acid fortification had been implemented). Subjects were considered Latino if they, their parents, or their grandparents were born in Mexico or Central or South America. Of the sample, 85% were of Mexican ancestry. The details of sampling and recruitment are described elsewhere (10).

Sample collection and analysis

Fasting blood was collected from each participant during home visits. Tubes were wrapped in foil, immediately put on ice,

and transported to the University of California, Davis, Medical Center Clinical Laboratory for processing within 4 h of collection. Plasma and serum were isolated and stored at −80 °C until analysis. Plasma folate and vitamin B-12 concentrations were measured by using radioassay (Quantaphase II; BioRad Diagnostics, Hercules, CA). The upper limit for the folate standard curve in this assay is 45.3 nmol/L. Subjects with concentrations above this upper limit were assigned a plasma folate value of >45.3 nmol/L. Plasma homocysteine concentrations were determined by HPLC with postcolumn fluorescence detection (11).

Depressive symptoms assessment

Depressive symptoms were assessed by using the Center for Epidemiologic Studies Depression Scale (CES-D; 12). This scale consists of 20 questions addressing 6 symptoms of depression, including depressed mood, guilt or worthlessness, helplessness or hopelessness, psychomotor retardation, loss of appetite, and sleep disturbance. Each question is scored on a scale of 0–3, and the total CES-D score ranges from 0 to 60. A score of ≥ 16 is considered to be indicative of significant depression (12).

Demographic variables and acculturation assessment

Self-reported age, sex, and years of education were recorded for each subject. The Acculturation Rating Scale for Mexican Americans–II (13) is a self-reported measure of how well a person has assimilated into American culture, as judged by such factors as language preference, food choices, entertainment choices, and social group preference. Individuals were scored on a scale of 0–37; a score of 0 indicated no acculturation, and a score of 37 indicated complete acculturation.

Supplement, prescription drug, and alcohol use

Supplement and prescription medicine use was ascertained by visual examination of medications: the type and exact dose were noted, and the medication type was compared with a standard pharmaceutical database. Subjects taking folic acid or a folic acid–containing multivitamin were included in the folic acid supplement group. Subjects taking selective serotonin reuptake inhibitors, tricyclics, monoaminoxidase inhibitors, and other atypical antidepressants were included in the antidepressant group. Women taking estrogen with or without progesterone were included in the hormone replacement therapy (HRT) group. By self-report, subjects indicated the consumption of alcohol, including beer, wine, and spirits. Subjects were divided into 3 groups of alcohol consumption, defined as "never or rarely" (never or yearly), "occasional" (weekly or monthly), and "often" (daily).

Statistical analysis

We recruited 1789 subjects at baseline for the SALSA study. Plasma folate concentrations were available for 1510 subjects, and complete data, including all covariates, were available for 1463 subjects. Subjects were categorized by sex and by low (CES-D score <16) or high (CES-D score $\geq$16) depressive symptoms. The difference in the percentages of men and women with a CES-D score $\geq$16 was assessed by chi-square analysis. Differences between men and women, differences between the low and high CES-D score groups, and potential CES-D score group × sex interactions were assessed by using two-factor analysis of variance on the ranks of age, years of education, acculturation score, CES-D score, and folate, vitamin B-12, and homocysteine concentrations. Differences between men and women, differences between the low and high CES-D score groups, and potential CES-D score group × sex interactions were assessed by using logistic regression analyses for folic acid supplement use and antidepressant use. Logistic regression models were generated also to assess the potential effect of plasma folate tertile × sex and plasma folate tertile × HRT use interactions on CES-D score. Additional logistic regression models were generated to assess associations between plasma folate tertile and low or high CES-D score in men and women separately. Both unadjusted and adjusted logistic regression models were produced; the latter controlled for potential confounding by age, education, acculturation, vitamin B-12 and homocysteine concentrations, folic acid supplement and antidepressant use, and alcohol consumption. Plasma folate concentrations were categorized into tertiles because the plasma folate measurements had a ceiling at 45.3 nmol/L, as described above. Values for plasma homocysteine and vitamin B-12 concentrations were positively skewed and nonnormally distributed. These variables were natural log transformed before analysis. Significance was defined for all analyses as $P < 0.05$. The statistical analyses were carried out by using STATVIEW for MACINTOSH (version 5.0.1; Apple Computer Co, Cupertino, CA).

RESULTS

A significant proportion of the SALSA study population (26%) had plasma folate concentrations >45.3 nmol/L, which is the upper limit of the standard curve for the folate radioassay. Only 0.3% of the population was folate deficient, which is defined as a plasma folate concentration $\leq$6.8 nmol/L. The low prevalence of folate deficiency is likely the consequence of the US government–mandated fortification of flour with folic acid since 1998 (7, 8). It is important that all subjects were recruited after the initiation of the folic acid fortification program.

The characteristics of the SALSA study population divided by depression score (CES-D score <16 or $\geq$16) and sex are presented in **Table 1**. Twenty-five percent of the total population had a CES-D score $\geq$16; a significantly ($P < 0.001$) higher percentage of the women than of the men had a CES-D score $\geq$16 (32% and 16%, respectively). The high CES-D group had significantly ($P \leq 0.04$) greater age, fewer years of education, lower acculturation scores, and lower plasma folate and higher vitamin B-12 concentrations than did the low CES-D score group (irrespective of sex). There were no significant differences between the CES-D score groups in total plasma homocysteine concentration, the percentage of subjects taking folic acid supplements, and the percentage of subjects taking antidepressant medications. The women also had significantly higher vitamin B-12 and lower homocysteine concentrations and a significantly higher proportion of antidepressant use than did the men, irrespective of CES-D scores ($P \leq 0.04$). No significant differences were observed between women and men with respect to age, education, acculturation score, plasma folate concentration, and folic acid supplement use. We also investigated whether there was any effect of CES-D score × sex interactions on any of the variables presented in Table 1. No significant interactions were observed except for plasma folate ($P = 0.03$).

TABLE 1
Characteristics of Sacramento Area Latino Study on Aging sample by sex and Center for Epidemiologic Studies Depression Scale (CES-D) score

| | Men | | Women | | P^1 | | |
| | CES-D score | | CES-D score | | | Low vs high | Sex × |
	<16 ($n = 527$)	≥16 ($n = 100$)	<16 ($n = 598$)	≥16 ($n = 285$)	Men vs women	CES-D score	CES-D score
Age (y)	68 (60–93)[2]	70 (60–87)	70 (60–93)	70 (60–92)	NS	0.04	NS
Education (y)	8 (0–25)	5 (0–16)	8 (0–24)	5 (0–32)	NS	<0.001	NS
Acculturation score (0–37)	19 (4–37)	16 (1–33)	19 (0–35)	14 (2–36)	NS	<0.001	NS
CES-D score (0–60)	3 (0–15)	21 (16–49)	4 (0–15)	24 (16–54)	0.04	<0.001	NS
Folate (nmol/L)	30.4 (6.1 to >45.3)	28.6 (9.3 to >45.3)	34.2 (6.3 to >45.3)	28.6 (5.2 to >45.3)	NS	0.03	0.03
Percentage with folate <6.8 nmol/L (%)	0.2	0	0.2	0.4			
Percentage with folate >45.3 nmol/L (%)	23.3	23	30.6	23.9			
Vitamin B-12 (pmol/L)	279 (16–1305)	304 (82–944)	313 (49–1452)	348 (63–1367)	<0.001	<0.001	NS
Homocysteine (μmol/L)	10.4 (4.4–129.2)	10.7 (6.5–115.4)	9.3 (4.7–65.8)	9.3 (4.2–36.3)	<0.001	NS	NS
Percentage taking folic acid supplements (%)	5.7	6.7	5.8	5.6	NS	NS	NS
Percentage taking anti-depressants (%)	3.5	7.6	7.6	13.4	0.006	NS	NS

[1] Two-factor ANOVA or logistic regression.

[2] Median; range in parentheses (all such values).

We therefore performed secondary analyses in which odds ratios (ORs) were evaluated as indicators of the strength and direction of the relation between plasma folate concentration and CES-D score for the men and the women. First, logistic regression models were developed to evaluate the effect of the sex × plasma folate tertile interactions (**Table 2**) on CES-D score. Sex was found to be independently associated with high CES-D score in the unadjusted model, but not in the adjusted model after control for potential confounding by age, education, acculturation, vitamin B-12 and homocysteine concentrations, folic acid supplement and antidepressant use, and alcohol consumption. The plasma folate tertile was not independently associated with a high CES-D score, but the interaction between sex and folate tertile was significant. This significant interaction was observed in both the unadjusted and adjusted models. Subsequently, separate logistic regression analyses were performed for the women and men (**Table 3**). Among the women, those in the lowest tertile of plasma folate had an adjusted OR for high CES-D score of 2.04 (95% CI: 1.38, 3.02; $P < 0.001$). In contrast, the men in the lowest tertile of plasma folate had an OR for high CES-D score that was not significantly different from unity.

Information on HRT use was available for 840 of the 883 women included in this analysis: 23% of the women were taking HRT, and ≈80% of that group were taking 0.625 mg Premarin/d (equine-based, unopposed estrogen; Wyeth-Ayerst Laboratories, Philadelphia; 14). Because HRT is known to affect mood (15) and because the association between depressive symptoms and plasma folate tertile was observed only in the women, a logistic regression analysis was performed to ascertain whether there was an effect of the interaction between HRT and plasma folate tertile on the CES-D score. No effect of HRT or of an interaction between HRT and plasma folate tertile was observed (data not shown).

DISCUSSION

This study is the first population-based study in the United States to assess the relation between folate status and depressive

TABLE 2
Odds ratios (ORs) for Center for Epidemiologic Studies Depression Scale score ≥16 for sex and low folate status

	OR (95% CI)[1]	P
Unadjusted model ($n = 1510$)		
Sex (women)	1.93 (1.21, 3.09)	0.006
Plasma folate		
(tertile 1)[2]	0.96 (0.56, 1.65)	0.887
(tertile 2)	1.14 (0.67, 1.93)	0.632
Sex (women) × plasma folate		
(tertile 1)[2]	2.17 (1.14, 4.12)	0.018
(tertile 2)	1.03 (0.54, 1.95)	0.928
Adjusted model ($n = 1463$)[3]		
Sex (women)	1.48 (0.90, 2.44)	0.122
Plasma folate		
(tertile 1)[2]	0.75 (0.42, 1.33)	0.322
(tertile 2)	0.96 (0.55, 1.67)	0.882
Sex (women) × plasma folate		
(tertile 1)[2]	2.75 (1.40, 5.40)	0.003
(tertile 2)	1.18 (0.60, 2.30)	0.627

[1] Determined by logistic regression analyses and are for women compared with men, folate tertile 1 (plasma folate 5.26–24.63 nmol/L) and folate tertile 2 (plasma folate 24.65–40.00 nmol/L) compared with folate tertile 3 (plasma folate >40.00 nmol/L), and interactions between sex and folate tertiles.

[2] Overall chi-square test P values for plasma folate tertiles in each model were 0.79 and 0.54 (unadjusted and adjusted). Overall chi-square test P values for the interaction between sex and plasma folate tertiles in each model were 0.02 and 0.005 (unadjusted and adjusted).

[3] Controlled for potential confounding by age, education, acculturation, ln(vitamin B-12), ln(homocysteine), folic acid supplements and antidepressant use, and alcohol consumption. Sample size (n) is less in the adjusted model because of missing values for one or more of the covariates.

TABLE 3
Odds ratios (ORs) for Center for Epidemiologic Studies Depression Scale score ≥16 for plasma folate tertiles divided by sex[1]

	OR (95% CI)[2]	P
Unadjusted models		
Women (n = 883)		
Tertile 1	2.08 (1.47, 2.95)	<0.001
Tertile 2	1.17 (0.82, 1.67)	0.383
Tertile 3	1.0 (reference)	
Men (n = 627)		
Tertile 1	0.96 (0.56, 1.65)	0.887
Tertile 2	1.14 (0.67, 1.93)	0.633
Tertile 3	1.0 (reference)	
Adjusted models[3]		
Women (n = 852)		
Tertile 1	2.04 (1.38, 3.02)	<0.001
Tertile 2	1.12 (0.76, 1.64)	0.581
Tertile 3	1.0 (reference)	
Men (n = 611)		
Tertile 1	0.74 (0.40, 1.35)	0.325
Tertile 2	0.99 (0.55, 1.76)	0.965
Tertile 3	1.0 (reference)	

[1] Tertiles 1–3 were 5.26–25.24, 25.25–42.24, and > 42.24 nmol/L for women and 6.05–23.70, 23.71–37.21, and > 37.21 nmol/L for men. Overall chi-square test values for plasma folate tertiles in each model were $P < 0.001$ (unadjusted and adjusted) for women and $P = 0.79$ (unadjusted) and $P = 0.50$ (adjusted) for men.

[2] Determined by logistic regression analyses.

[3] Controlled for potential confounding by age, education, and acculturation, ln(vitamin B-12), ln(homocysteine), folic acid supplements and antidepressant use, and alcohol consumption. Sample sizes (n) are less in the adjusted models because of missing values for one or more of the covariates.

symptoms in the era of folic acid fortification. The data indicate that, despite folic acid fortification and independent of age, education, acculturation, vitamin B-12 and homocysteine concentrations, folic acid supplement and antidepressant use, and alcohol consumption, low plasma folate is associated with depressive symptoms in elderly Latina women (but not elderly Latino men).

Before folic acid fortification, several population-based studies assessed the relation between folate status and depression in the United States (16–18). Consistent with the present study, Morris et al (16) found, using data from the third National Health and Nutrition Examination Survey (data collected from 1991 to 1994), that young adults (aged 15–39 y; n = 2948) with symptoms of major depression had lower plasma folate concentrations than did those without depressive symptoms. In contrast, Lindeman et al (17) found no relation between folate status and depressive symptoms in the New Mexico Elder Health Survey of older adults (aged ≥65 y; n = 833; data collected 1993–1995). Penninx et al (18) also found no relation between folate status and depressive symptoms in physically disabled older women (aged ≥65 y; n = 700) participating in the Women's Health and Aging Study (data collected 1992–1995). It is unclear why these latter 2 studies are inconsistent with the SALSA study, particularly because they were carried out before the implementation of folic acid fortification, during a time when the relation between folate status and depression might have been expected to be stronger. One important difference is that those 2 studies used the Geriatric Depression Scale (19) to assess depressive symptoms, whereas the SALSA study used the CES-D scale.

Additional studies have assessed the relation between folate status or intake and depressive symptoms in European populations without exposure to folic acid fortification (20–22). Bjelland et al (20) found in a Norwegian study (n = 5948) that low plasma folate was associated with elevated depressive symptoms in middle-aged women (aged 45–49 y), but not in elderly women (aged 70–74 y) or in middle-aged or elderly men. Depressive symptoms were assessed in that study by using the Hospital Anxiety and Depression Scale (23). Notably, Bjelland et al (20) also observed that the common $677C \rightarrow T$ polymorphism in the folate-metabolizing enzyme, methylenetetrahydrofolate reductase (MTHFR), was associated with elevated depressive symptoms. We have not assessed the *MTHFR* genotype in the SALSA study, but it is possible that it is an important determinant of depressive symptoms in this population, because the homozygous "TT" isoform is highly prevalent in Latinos (24).

In another study carried out in Rotterdam (Netherlands), Tiemeier et al (21) found no relation between folate status and depressive symptoms assessed by using the CES-D scale in community-dwelling older adults (aged ≥55 y; n = 694). A notable difference between the Rotterdam study and the SALSA study is the ways in which folate status was characterized. In the Rotterdam study, folate deficiency was defined as a plasma folate concentration <5.0 nmol/L and a total plasma homocysteine concentration >13.9 μmol/L. Because elevated homocysteine is not specific to folate deficiency (homocysteine can be elevated because of vitamin B-12 and vitamin B-6 deficiencies, renal disease, and hypothyroidism, among other potential factors), this definition potentially may have confounded the results of the Rotterdam study. Specifically, subjects who were, in fact, folate deficient but who did not present with hyperhomocysteinemia would have been included in the folate-replete group, which would reduce the investigators' ability to observe a significant relation between folate status and depressive symptoms. We performed a similar analysis in the SALSA study population and found that the combination of low folate (defined as the lowest tertile of plasma folate) and elevated plasma homocysteine (defined as >13 μmol/L) concentrations was not a significant predictor of elevated depressive symptoms (data not shown). We also found that an elevated homocysteine concentration alone was not associated with depressive symptoms. This is inconsistent with the findings of Bjelland et al (20), who showed a significant association between hyperhomocysteinemia and elevated depressive symptoms, but it is consistent with the findings of Fava et al (2), who did not show an association in 213 patients with major depressive disorder. Again, discrepancies among studies may be due, in part, to differences in the instruments used to assess depressive symptoms. Differences in the national and ethnic distribution of the study populations and in other demographic or socioeconomic factors may also be important.

One additional European study looked at the association of dietary folate intake and depressive symptoms. Tolmunen et al (22) found a significant association between low folate intake and elevated depressive symptoms in a cohort of middle-aged Finnish men (n = 2682; aged 42–60 y). They used the Human Population Laboratory Depression Scale (25).

The association between folate status and depressive symptoms in the SALSA study was observed only in the women. A contributing factor may be that all the women in that study are postmenopausal. Previous studies showed that postmenopausal women are at elevated risk of depression (26, 27). Moreover,

some studies have shown that HRT reduces the depressive symptoms that may occur after menopause (15). In the present study, no significant effect of the interaction between folate status and HRT on depressive symptoms was observed in the women, which indicates that the relation between low folate status and depressive symptoms is independent of HRT use. Our additional finding that the median plasma vitamin B-12 concentration was higher in the high CES-D score group than in the low CES-D score group is somewhat surprising. At least 2 studies have found that low vitamin B-12 status is associated with depressive symptoms (2, 21). This discrepancy remains to be explained.

In conclusion, elderly Latina women may have an increased requirement for folate to prevent or minimize depressive symptoms. To test this hypothesis, intervention studies investigating the effect of folic acid on depressive symptoms in elderly women with low or low-normal folate status should be conducted.

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